VISUALIZATION OF INTRACELLULAR ICE CRYSTAL FORMATION USING X-RAY MICRO-COMPUTED TOMOGRAPHY

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ABSTRACT

This study demonstrates the capability of X-ray Micro-computed Tomography (XMT) technique to characterise the internal ice crystal microstructure of freeze-dried samples (strawberry). The method requires the fruit being frozen under specific freeze-drying temperature of the samples to remove frozen water before scanning to indicate ice crystal and internal structure of the samples. Results are presented for the 2-D ice crystals formed within the samples. The dendrite spacing (size, volume and width) of ice crystals is related to freezing condition of the samples. Only 1% small intercellular voids distribution of the total 99% volume in fresh strawberry samples as compared to 72% void or pore distribution in freeze-dried samples. Other than that, the average width size of ice crystal for two different operating freezing (-20^{o}C and -80^{o}C) temperatures for strawberry samples was 0.15 to 0.29 mm and 0.29 to 0.40 mm respectively. The overall results indicate that the ice crystal distribution within samples were diverse with the axial distance of the material from its cooling surface. The ice crystal size is bigger when the fruits were far from the cooling surface. At the investigated conditions, the comparisons with air-drying technique had been done on jackfruit and the study on those fruits had been limited to image visualization for validation and nutrient analysis. Samples dried in the freeze dryer were seen to retain their shape much better than air dried samples which underwent shrinkage and change in color. Samples dried in freeze dryer possessed less dense structures and consequently displayed more favorable rehydrated textural properties than the air-dried equivalents. Furthermore the total phenolic (TP), and ascorbic acid (AA) of the fresh and freeze-dried samples had been investigated. This method had preserved 3.65 mg/mL AA concentration in freeze-dried strawberry samples with ultrasonic pre-treatment and no AA had been detected in the air-dried strawberry samples. Total phenolics (TP) concentrations was also evaluated and compared to the TP content in fresh samples that were frozen and stored at -20^{o}C. The average TP content of frozen, freeze-dried and air-dried strawberries are 270.5, 231.0, and 28.7 mg/100 g of fresh weight, respectively.

ABSTRAK

Kajian ini menunjukkan kemampuan teknik X-ray mikro tomografi (XMT) untuk mencirikan secara mikro ais kristal yang terbentuk di dalam sampel beku-kering (strawberi). Kaedah ini memerlukan sampel buah yang dibekukan di bawah suhu beku-kering tertentu untuk menghapuskan kesan ais sebelum imbasan untuk menunjukkan hablur ais dan struktur dalaman sampel dijalankan. Keputusan dipaparkan secara 2-D untuk ais kristal yang terbentuk di dalam sampel buah beku-kering. Jarak dendrit ais kristal (saiz, isipadu dan luas) dipengaruhi oleh kaedah pembekuan terhadap buah-buahan. Hanya 1% ruang interselular pengedaran didapati dalam 99% jumlah sampel (strawberry segar) berbanding dengan ruang 72% pengedaran pori di dalam sampel beku-kering (strawberry). Selain itu, saiz ais kristal bagi sampel yang dibekukan pada -20^{o}C dan -80^{o}C sebelum menjalani beku-kering teknik adalah 0.15-0.29 dan 0.40-0.29 mm. Keputusan keseluruhan menunjukkan bahawa pelbagai bentuk taburan ais kristal di dalam sampel berbeza mengikut jarak sampel dari permukaan pendinginnya. Saiz ais kristal lebih besar apabila sampel buah berada jauh dari permukaan pendingin. Pada keadaan diselidiki, perbandingan dengan pengeringan secara pemanasan dijalankan ke atas buah nangka dan keputusan kajian adalah terhad kepada paparan imej dan analisis nutrien sahaja. Sampel yang dikeringkan menggunakan alat beku kering ternyata jauh lebih baik untuk mempertahankan bentuk asal berbanding pengeringan secara pemanasan dimana ia mengalami perubahan penyusutan bentuk dan warna. Sampel yang dikeringkan dalam alat beku kering mempunyai struktur kurang padat berbanding pengeringan secara pemanasan. Selain itu, kandungan total fenol (TP), dan asid askorbat (AA) sampel segar dan beku-kering telah dianalisis. Keputusan telah menunjukan 3.65 mg/ml AA masih terkandung dalam sampel beku-kering menggunakan kaedah ultrasonik dan AA tidak dapat dikesan di dalam sampel yang melalui pengeringan secara pemanasan. Kandungan TP pada sampel segar yang dibekukan dan disimpan pada-20^{o}C juga dibandingkan. Purata kandungan TP bagi teknik secara beku, beku-kering dan pengeringan biasa buah strawberi adalah 270.5, 231.0, dan 28.7 mg/100 g.

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LIST OF ABBREVIATIONS

Micro-CT	Micro-computed tomography
CTan	Skyscan tomographic analysis package
°C	Degree Celsius
°F	Degree Fahrenheit
2-D	Two-dimensional
3-D	Three-dimensional
mm	Millimetre
g	Gram
μA	Current(microampere)
kV	Voltage(kilo volt)
mTorr	Pressure (mili Torr)
%	Percentage
g/ml	Concentration(gram/millilitre)

CHAPTER 1

INTRODUCTION

1.1 RESEARCH BACKGROUND

Improving methods of food preservation is an essential technique needed by all in the food industry. Achieving the right preservation technique is very important in relation to the changes of nutritional and sensory qualities of food. Freezing is a conventional food preservation technique in which the temperature of food is reduced below its freezing point and a proportion of the water undergoes a change in its state to form ice crystals. The immobilization of water to ice and resulting concentration of dissolved solutes in unfrozen water lowers the water activity of the food (Fellows, 2000). This method often results in substantial textural damage caused by the growth of ice crystals within the delicate structure either present naturally or created during processing (Mousavi et al., 2005).

The preservation of biological products by reducing their water content can be achieved by several dehydration techniques. The underlying principle of drying foods is to lower the moisture content in order to reduce water activity and prevent spoilage. Water activity is a critical factor that determines shelf life, with most bacteria unable to grow below a value of 0.91 and moulds cease to grow below 0.80 (Brown et.al., 2008 and Beuchat, 1983).Water activity also plays a significant role in determining the activity of enzymes and vitamins in foods and can have a major impact on their colour, taste and aroma. Additionally, moisture removal reduces the weight and the bulk of food products to facilitate transport and storage. While imparting these benefits, loss of moisture during drying may also inflict undesirable effects on the product's microstructure (Brown et al., 2008). This is unfortunate given that food structure influences nutritional availability, chemical and microbiological stability, texture, physical properties and transport properties (Aguilera, 2005; Aguilera et al., 2003; De Roos, 2003 and Aguilera et al., 2000).

Among these methods, freeze-drying is considered as the reference process for manufacturing high-quality dehydrated products. This drying process involved a preliminary freezing of the products followed by placing them under reduced pressure (< 300 Pa) with a sufficient heat supply to sublimate ice (2800 J per gram of ice). Compared to classical dehydration techniques, the main advantages of the freeze-drying process are: (i) the preservation of most of the initial raw material properties such as shape, appearance, taste, colour, flavour, texture, and biological activity and (ii) the high rehydration capacity of the freeze-dried product (Hammami and Rene, 1997). Freeze-drying is a technique that results in high-quality dehydrated products due to the absence of liquid water and the low temperatures required in the process. The solid state of water during freeze-drying protects the primary structure and minimizes changes in the shape of the product, with minimal reduction of volume (Ratti, 2001). In addition, it contributes to preserve constituents such as minerals and vitamins, as well as to retain original flavour and aroma (George et al., 2002).

Freeze-drying appears, therefore, as a promising technique for dehydration of thermal-sensitive materials, such as strawberries and jackfruits. The final quality of dried fruits can be affected by the physical and structural changes that occur during drying, which may include deformation in terms of shrinking. The true and apparent densities, as well as the porosity of the material define the rehydration capacity of a dried product. The drying process may alter these properties, resulting in products with modified texture, optical, thermal and nutritional properties (Krokida et al., 2000).

Another parameter that can be used as a quality index of nutrients during food processing and storage is the ascorbic acid content, due to it being an unstable constituent, sensitive to variations on pH, temperature, moisture content, oxygen and light. If the ascorbic acid is retained after processing, other nutrients are likely to be retained. Lin et al. (1998) performed a comparative study among vacuum microwave, hot air and freeze-dried carrot slices. They observed that freeze-dried carrots did not present significant losses in the content of Vitamin C, while the samples dried by hot air and vacuum microwave presented losses of 62% and 21%, respectively. Luanda et al. (2006) and Shadle et al. (1983) also investigated the Vitamin C content of carrots after convective and freeze-drying, reported losses of 81.3% and 60.8%, respectively in the dry samples.

The rehydration ratio can be considered as a measure of the injuries caused by the processing and drying to the material (Krokida et al., 2003). It is generally accepted that the rehydration capacity is dependent on the degree of cellular and structural disruption. During drying, Jayaraman et al. (1990) observed irreversible cellular rupture and dislocation, resulting in loss of integrity and hence, in a dense structure of collapsed, greatly shrunken capillaries with reduced hydrophilic properties, which are reflected by the inability to imbibe sufficient water to fully rehydrate.

This study is concerned within the freeze-drying for strawberry. The aim was to determine the influence of processing conditions on the ice crystal formation in those fruits by using the application of X-ray Micro-computed Tomography (XMT) techniques. Ice crystal growth in fruits has been found to extensively damage those fruits. Samples of strawberry were selected because they are highly perishable fruit with very short life span (not more than 4 to 5 days in refrigeration conditions). In spite of the importance of knowing the ice crystal formation of these freeze-dried fruits, detailed information on visualization is still lacking in the literature, particularly for frozen fruits. The XMT techniques applicable in this study had been done by collaborated with Malaysian Nuclear Agency (MINT) in which provided the equipment for data collection. The purpose of this work is also to determine quality parameters, such as the ascorbic acid (AA), total phenolic(TP), the glass transition temperature, the water activity and the rehydration capacity for freeze-dried samples and to investigate the effect of dehydration conditions on these properties. The nutrient analysis had been done on collaboration with CEPP, Univesiti Teknologi Malaysia and SIRIM QAS International Sdn. Bhd.

1.2 PROBLEM STATEMENT

A major problem with freeze-drying process is the preliminary freezing of the product will stiffen its structure and subsequently prevent solute and liquid motion during freeze-drying (Hammami and Rene, 1997, and Levine and Slade, 1989). During the formation of ice crystals, they grow and create a uniform network throughout the product that after sublimation yields a dense, spread and homogeneous porous matrix.

Chemical and enzymatic reactions will thus be significantly limited and the phenomena of aroma loss and vitamin degradation will be reduced in comparison to classical drying techniques (Hammami and Rene, 1997, and Simatos et al., 1974). The sublimation phenomenon (direct change from ice to vapour) explains the reason freeze-dried products adulterate a little or not at all and can rehydrate instantaneously. The poor quality and alterations of freeze-dried products that are sometimes encountered are generally linked to the quality of the raw material (nature and degree of ripeness) and to processing conditions (operating pressure, heating temperature, freezing rate, freeze-drying process control) (Genin and Rene, 1996).

Previously a number of research efforts had been reported on the ice crystal formation in frozen food (such as fish, meat and mycoprotein) that may affect their textural, microstructural and qualitative changes. The study on quality of freeze dried strawberry pieces had been done by Hammami and Rene (1997). They had conducted an experiment on a thick layer of strawberry pieces in different operating conditions of freeze drying. Researchers found that working pressure and heating plate temperature during freeze drying were the most important factors affecting the criteria of the final product quality in terms of its appearance, shape, colour, texture and dehydration ratio. The researchers had found the optimal conditions for freeze drying process for strawberry was at 30 Pa, 50^{o}C and the time ranged for freeze drying was from 60 to 65 hours. Although the researchers had investigated the freeze drying time, appearance and colour of the freeze dried strawberries, there was no qualitative and quantitative information about ice crystal formation of these strawberries in thick samples.

Recent studies visualized the ice crystal structures formed during freezing of a number of foods had been applied using X-ray micro-computed tomography (XMT) (Mousavi et al., 2007). An understanding of the relationship between the freezing conditions and the size of ice crystals formed is critical in controlling product quality and texture (Mousavi et al., 2007). The observation of the ice crystals size can be direct or indirect. Direct observation of ice crystal can be done by cryo-scanning electron microscope (Russell et al., 1999), cold microscopy (Donhowe et al., 1991) and confocal laser scanning microscopy (Evans et al., 1996).

While the indirect method such as freeze substitution (Bevilacqua et al., 1979, and Martino and Zarizky, 1998), freeze fixation (Miyawaki et al., 1992) and freeze drying techniques (Woinet et al.,1998a, b; and Fayadi et al.,2001) which followed by the sectioning had also been used. However, the indirect methods assumed the original morphology is maintained during the sectioning into thin enough layers to allow microscopic methods to be used (Mousavi et al., 2005).

Freeze drying is a well established process for the indirect method to observe ice crystals formation and preserve the food products. Freeze-drying is known to produce products of excellent quality, allowing significant structural preservation (Brown et al., 2008 and Sinesio et al., 1995). Indeed it has been reported that the products of freeze-drying are of higher porosity than those of air-drying (Brown et al., 2008; Karathanos et al., 1996; Marabi and Saguy,2004, and Rahman et al., 2002) and other drying methods such as microwave (Brown et al., 2008 and Tsami et al., 1999), and vacuum-drying (Rahman et al., 2002).

X-ray micro-CT (XMT) is relatively a new technique which has found potential applications in food science research and quality evaluation (Mendoza et al., 2006). An axial and lateral resolution down to a few micrometers and without sample preparation and chemical fixation of the architecture of cellular materials can be visualized and analyzed through this technique (Dalen et al., 2003). Studies had been done using XMT. They were firstly studies on the internal microstructure for ice crystal visualization of mycoprotein, carrot, meat, fish, chicken, potato and cheese (Mousavi et al., 2005, 2007), secondly studies to detect internal quality changes in peaches (Barcelon et al.,1999), thirdly investigation on core breakdown disorder in ‘Conference’ pears based on their mass density variations during storage (Lammertyn et al., 2003) and lastly quantitative analyze and characterization of apple tissue to micrometer resolution (Mendoza et al., 2006). X-rays are short wave radiations, which can penetrate through fruit (Mendoza et al., 2006). The level of transmission of these rays depends mainly on the mass density and mass adsorption coefficient of the material (Mendoza et al., 2006 and Salvo et al., 2003). The density of many fruits increases with maturity (Mendoza et al., 2006 and Baoping, 1999).

The motivation of those studies was found in the necessity to extract realistic and statistical 2-D internal data for the ice crystals formation in freeze-dried strawberry at micron resolution and thus validate those results by using another fruits for comparison and jackfruit had been selected in order to make a comparison of micro structural evaluation and nutrient analysis with strawberries. Observing the ice crystals formation of the internal microstructure of food is important as it will help in preserving the texture quality of the freeze-dried strawberry and jackfruit.

The criterion of quality is becoming progressively more important in consumer choice. Thus, industrial products and ingredients must offer different convenient properties (tasty, healthy, and safety) that are close to those of fresh product. At the same time, new market demands are emerging that could concern freeze-dried products, for example dehydrated fruits to add in cereals, cereal bars, ice creams, or pastries.

1.3 OBJECTIVE

The main objectives of this study are:

i. to investigate the potential of freeze-drying techniques for strawberry and jackfruit according to the experimental set up,
ii. to compare freeze-dried and conventional air-drying techniques on those fruits and the results were subjected only to image validation using XMT and nutrient analysis,
iii. to employ the application of XMT techniques in order to study the extent of the damage produced by ice crystal growth in freeze-dried strawberry fruit tissues in more details and,
iv. to analyze the nutrient constituents of those freeze-dried fruits. The constituents of ascorbic acid (AA), total phenol (TP), colour, appearance and rehydration had been investigated in those fruits.

1.4 SCOPE

The research is based on experimental studies of freeze-drying technique for fruits by using strawberry. In order to achieve the objectives mentioned earlier, five scoped had been investigated:

i. freeze-drying of strawberry at various operating conditions according to the experimental design: A study of different thickness (5mm, 10mm and 15 mm), ultrasonic treatment, and normal refrigeration freezer under $4^{o}C$, freezing at $-20^{o}C$ and $-80^{o}C$,
ii. comparison of freeze-drying with the conventional air-drying technique: using jackfruit as compared fruit and the result were evaluated only on image visualisation for ice crystal validation using XMT techniques and nutrient analysis.
iii. a study on MR images analysis from previous study in comparison with XMT image analysis in these studies.

iv. a micro-computed tomography scanner is used as characterisation device for the porosity changes while freeze- and air-drying is taking place. XMT device had been provided by Malaysian Nuclear Agency (MINT) for the use of data collections. This scanner uses up-to-date scanning techniques to take x-ray photographs of the sample, while the latter is rotated perpendicularly about its long axis. Reconstruction software then recreates the photographs as circular planes and the extent of drying on the surface of the strawberry sample can be assessed by the amount and size of the pores on its surface for different applicable pre-treatment, thickness and nutritional availability of the strawberry samples, and,

v. nutrient analysis of the fresh and freeze-dried or rehydrated strawberries and jackfruits (total phenol, and ascorbic acid content) using ultra violet (UV) spectrophotometer and high pressure liquid chromatography (HPLC) analysis. This work is also to determine some other quality parameters, such as the glass transition temperature, the water activity and the rehydration capacity for freeze-dried strawberries and to investigate the effect of dehydration conditions on these properties. Some parts of the evaluation (nutrient composition) had been done by collaboration with CEPP Universiti Teknologi Malaysia and SIRIM QAS International Sdn. Bhd.

1.5 SIGNIFICANT OF THE STUDY

Malaysia is one of the fruit producers, consumers and exporters of strawberry and jackfruit. Like other fruits, strawberry and jackfruit may be commercialized in their natural form, frozen pulps or processed juices. The typical weather characteristics that pre-dominate the northeast region in Malaysia with high relative humidity and temperatures however are not favourable to fruit preservation under natural conditions. Particularly for fruits with high moisture contents (in the case of strawberry, the average value is about 91%w.b.), rapid deterioration is commonly observed after cropping. The study also will offer an alternative method for preservation of the fruits and their original constituents. Other than that, special attention has been directed to the development of adequate drying techniques. Besides aggregating commercial value to the fruits, drying reduces wastes and post-harvest losses, and might allow their commercialization for extended periods, with minor dependence on seasonal conditions.

Freeze-dried preservation retains the nutrient quality of agricultural products over long storage periods. As a method of long-term preservation for fruits and vegetables, freeze-dried is generally regarded as superior to canning and dehydration, with respect to retention in sensory attributes and nutritive properties (Fennema, 1973). The research in this field will be very useful to the frozen industry. The need for freeze-dried food products had become one of the most in demand technologies in food preservation. The significances of this study are:

i. Strawberry and jackfruit itself had been selected as the main raw material in this study due to the existing of many natural antioxidants properties in those fruits and thus giving more health benefits to the consumers. As an example, ellagic acid that was mostly found in strawberry had been useful antioxidant to prevent cancer. Other than that, there is still no added value to those fruit in our local market as an example to produce food product with existing of this natural fruits.
ii. It is important to study the composition of different foods in order to understand the nature of those foods. The nature of the food like color, texture, consistency and quality depends on the constituents that contain in it. This study will help in eliminates the use of synthetic coloring and flavoring agent in food products by introducing the freeze-dried techniques that can help in preserving the initial properties in foods.
iii. Understanding the changes that occur in food during storage, preparation and processing. Food is exposed to different conditions during storage, preparation and processing and these changes may either be desirable or undesirable. The knowledge of undesirable changes during storage, preparation and preparation will enable us to develop and use ideal methods of food storage, preparation and processing which would retain the nutrients to the maximum along with increasing the acceptability.
iv. The study also aiming to offer alternatives fruits preservations which is freeze-drying technique that can make the process more economically (no chemical added to the raw material in this continous processes and

produce products that retains most of its initial properties), environmental and consumer friendly (no more waste from fruits and have fruits on their diets plans) and the design is compact and easy to operate in the food industry.

v. Other than that, the significance of having new types of fruits products which can give longer shelf life, reduced weight for storage, shipping and handling become easier in order to commercialize this new product (freeze-dried) fruits. Our food industry will no more dependable on the seasonal condition and thus will help them in producing new foods (cereals, snack bars) which includes the original fruit taste that can change the consumer bad eating habit to have a portion of fruits in their diet plans.

vi. XMT also can contribute in designing optimized production for freeze-drying products as it will help in observing at micron resolution the internal microstructure of the food products in order to give more preferable freeze-dried products.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

The prime interest in the literature study was the use of x-ray micro-computed tomography (XMT) techniques in analyzing the influence of processing conditions (working pressure and heating temperature) on freeze-drying time and product microstructure and, to a lesser extent, the shelf-life of the freeze-dried products and the changes in some properties during storage (Hammami and Rene, 1997; Roos, 1987, and Paakkonen and Mattila, 1991). Significant progress was reached in the understanding of the phenomena occurring during freeze-drying (Hammami and Rene, 1997; Monteiro-Marques et. al., 1991; Rutledge et al., 1994, and Genin and Rene, 1996), quality improvement of freeze-dried vegetables (Hammami and Rene, 1997; Le Loch et al., 1992; Kompany and Rene, 1993; Genin and Rene et al., 1996) or the monitoring and controlling of the freeze-drying process (Hammami and Rene, 1997; Rene et al., 1993, and Roy and Pikal, 1989).

In some cases, improvement (energy saving and quality assessment) of classical dehydration techniques such as hot air drying, fluidized bed drying, vacuum drying, and spray-drying (Hammami and Rene, 1997, and Mujumdar, 1987), as well as the development of new techniques such as ‘puffing’ (Hammami and Rene, 1997, and Guimard, 1994) had given rise to cheaper products that can be used in place of freeze-dried products. As a result, the challenges and opportunities for the freeze-drying technique require: (i) improvement of the quality of dehydrated products, and (ii) the processing of other products that are technically difficult to dehydrate at high temperature, as an example because of their high sugar content.

2.2 APPLICATION OF PRESERVATION TECHNOLOGY IN FOOD INDUSTRY

Food technology may be defined as a controlled attempt to preserve, transform, create or destroy a structure that has been imparted by nature or processing (Aguilera and Stanley, 1999). Other than that it is the application of science to the commercial processing of foodstuffs. Food is processed to make it more palatable or digestible, for which the traditional methods include boiling, frying, flour-milling, bread-, yoghurt-, cheese-making and brewing (Peter and Julian,1999). Food is also being processed to prevent growth of bacteria, moulds, yeasts, and other microorganisms. It is preserved from spoilage caused by the action of enzymes within the food that changes its chemical composition, resulting in changes in flavor, odour, colour, and texture of the food (Gary, 2008). These changes are not always harmful or undesirable; examples of desirable changes are the ripening cream in butter manufacture, the flavoring development of cheese, and the hanging of meat to tenderize the muscle fibres. Fatty or oily foods suffer oxidation of the fats, which makes them rancid (Peter and Julian, 1999).

Preservation enables foods that are seasonally produced to be available all year long. Traditional forms of food preservation include salting, smoking, pickling, drying, bottling, and preserving in sugar (Zeuthen and Bogh-Sorensen, 2003). Modern food technology also uses many novel processes and additives, which allows a wider range of foodstuffs to be preserved. All foods undergo some changes in quality and nutritional value when subjected to preservation processes. No preserved food is identical in quality compared to its fresh counterpart; hence only food of the highest quality should be preserved.

In order to grow, bacteria, yeasts, and moulds need moisture, oxygen, a suitable temperature, and food. The various methods of food preservation aim to destroy the microorganisms within the food, to remove one or more of the conditions essential for their growth. Adding large amounts of salt or sugar reduces the amount of water available for microorganisms to thrive because the water tied up by these solutes cannot be used for microbial growth (Barbosa-Canovas et al., 2008). This is the principle in salting meat and fish, and in the manufacture of jams and jellies. These conditions also inhibit the enzyme activity in food. Preservatives may also be developed in the food by controlling the growth of microorganisms to produce fermentation that make alcohol, acetic or lactic acid. Examples of food preserved in this way are vinegar, sour milk, yoghurt, sauerkraut, and alcoholic beverages (Peter and Julian, 1999, and Zeuthen and Bogh-Sorensen, 2003).

2.3 PRESERVATION TECHNOLOGY

Preservation enables foods that are seasonally produced to be available all year long. There are various types of preservation technology in food industry.

2.3.1 Refrigeration

At 5^{o}C (41^{o}F) or below 3^{o}C (37^{o}F) it will help in reducing spoilage for cooked foods, but it is less effective for foods with high water content. This process cannot kill microorganisms, nor stop their growth completely, and a failure to realize this limitation causes many cases of food poisoning. Refrigerator temperatures should be checked periodically as the efficiency of the machinery declines with age as higher temperature will be dangerous for the consumption of the refrigerated food. (Peter and Julian, 1999)

2.3.2 Deep Freezing

Deep freezing (-18^{o}C or below) stops almost all spoilage processes, except residual enzyme activity in uncooked vegetables and most fruits, which are blanched (dipped in hot water to destroy the enzymes) before freezing (Cano and Barta, 2008). Microorganisms cannot grow or divide while frozen, but most remain alive and can resume activity once defrosted. Preservation by freezing works by rendering water in foodstuffs unavailable to microorganism by converting it to ice. Some foods are damaged by freezing, notably soft fruits and salads, the cells of which are punctured by ice crystals, leading to the loss of crispness (Cano and Barta, 2008). Fatty foods such as cow’s milk and cream tend to separate.

Freezing has little effect on the nutritive value of foods, though a little vitamin C may be lost during blanching process in fruits and vegetables. Various processes are used for deep freezing foods commercially (Peter and Julian, 1999).

2.3.3 Pasteurization

It is used mainly for milk. By holding the milk at 72^{o}C or 161.6^{o}F for 15 seconds, all disease-causing bacteria can be destroyed (Doona and Feeherry, 2008). Less harmful bacteria survive, so the milk will still go sour within a few days (Peter and Julian, 1999).

2.3.4 Ultra-heat Treatment

It is used to produce UHT milk. This process uses higher temperatures than pasteurization, and kills all bacteria present, giving the milk a longer shelf life but altering the flavor (Doona and Feeherry, 2008).

2.3.5 Drying

It is effective because both microorganisms and enzymes need water to be active. This is one of the oldest, simplest, and most effective ways of preserving foods. In addition, drying concentrates the soluble ingredients in foods, and this high concentration prevents the growth of bacteria, yeasts and moulds. Dried food will deteriorate rapidly if allowed to become moist, but provided they are suitably packaged; products will have a longer shelf life. Traditionally, foods were dried in the sun and wind, but commercially today, products such as dried milk and instant coffee are made by spraying the liquid into a rising column of dry, heated air; solid foods, such as fruit, are spread in layers on a heated surface (Peter and Julian, 1999).

2.3.6 Freeze-drying

This technique is carried out under vacuum. It is less damaging to food than straight forward dehydration in the sense that foods reconstitute better. It is used for quality instant coffee and dried vegetables. The foods are fast frozen, and then dried by converting the ice to vapor under very low pressure. The foods lose much of their weight, but retain the original size and shape. They have a sponge like texture, and rapidly reabsorb liquid when reconstituted (Hamammi and Rene, 1997). Refrigeration is unnecessary during storage; the shelf life is similar to dried foods, provided the product is not allowed to become moist. The success of the method is dependent on a fast rate of freezing, and rapid conversion of ice to vapor. Hence the most acceptable result is obtained with thin pieces of food, and the method is not recommended for pieces thicker than 3cm. Fruit, vegetables, meat, and fish have proved to be satisfactory. This method of preservation is commercially used but the products are most often used as constituents of composites dishes, such as packet meals (Peter and Julian, 1999).

2.3.7 Canning

It relies on high temperatures to destroy microorganism and enzymes. The food is sealed in a can to prevent contamination. The effect of heat processing on the nutritive value of food is variable. For instance, the vitamin C content of green vegetables is much reduced, but owing to greater acidity in fruit juices vitamin C is quite well retained. There is also a loss of 25-50% of water soluble vitamins if liquor is not used. Vitamin B (thiamine) is easily destroyed by heat treatment, particularly in alkaline conditions. Acid products retain thiamine well, because they require only minimum heat during sterilization(Tewari and Juneja, 2008). The sterilization process seems to have little effect on the retention of vitamins A and B2. During storage of canned foods, the proportion of vitamin B and C decrease gradually. Drinks may be canned to preserve the carbon dioxide that makes them fizzy (Peter and Julian, 1999).